



What does engram encode?: Heterogeneous memory engrams for different aspects of experience

Miyu F. Nambu¹, Yu-Ju Lin¹, Josefine Reuschenbach¹ and Kazumasa Z. Tanaka





Abstract

Long-lasting synaptic changes within the neuronal network mediate memory. Neurons bearing such physical traces of memory (memory engram cells) are often equated with neurons expressing immediate early genes (IEGs) during a specific experience. However, past studies observed the expression of different IEGs in non-overlapping neurons or synaptic plasticity in neurons that do not express a particular IEG. Importantly, recent studies revealed that distinct subsets of neurons expressing different IEGs or even IEG negative-(yet active) neurons support different aspects of memory or computation, suggesting a more complex nature of memory engram cells than previously thought. In this short review, we introduce studies revealing such heterogeneous composition of the memory engram and discuss how the memory system benefits from it.

Addresses

Okinawa Institute of Science and Technology Graduate University (OIST), 1919-1 Tancha, Onna-son, Kunigami-gun, Okinawa, 904-0495, Japan

Corresponding author: Tanaka, Kazumasa Zen (kazumasa.tanaka@oist.jp)

 (Nambu M.F.),  (Lin Y.-J.),  (Reuschenbach J.),  (Tanaka K.Z.)

¹ Co-first authors.

philosophers for centuries. In 1904, Richard Semon brought up the concept of memory engrams [1]. He defined an engram as a physical change in the brain introduced by a particular experience. After encoding, specific cues can reactivate this engram leading to memory retrieval (ecophry). Despite highly systematic attempts to find the engram, the lack of certain technologies made it very difficult for Lashley and others to find evidence of long-lasting changes in the brain that represent particular experiences (memory trace) during the 20th century [2,3]. However, the progress in methods of labeling and manipulating specific cell groups helped find strong support for the existence of neuronal populations that contain the engram, so-called memory engram cells [4]. Josselyn and Tonegawa [5] describe three major engram evidence strategies: First, finding the activity of the same neurons during encoding and retrieval; second, manipulating this respective network of cells and investigating if this can cause retrieval (gain of function) or suppress (loss of function) respective behavioral output; third, implanting a memory by mimicking the encoding and retrieval process.

Activity-dependent neuronal tagging during a certain experience makes immediate early genes (IEGs) valuable for engram studies. Guzowski et al. (1999) enabled a significant step forward in this approach. They investigated the reactivation of a cell group during retrieval at two sequential time points by utilizing the distribution time of *Arc* mRNA from the nucleus to the cytoplasm [6]. Barth et al. (2004) sophisticated the IEG tagging technique. They developed a transgenic mouse line expressing a fosGFP fused protein under the *c-Fos* promoter [7]. This transgenic mouse line allowed immediate identification of c-Fos expressing cells and their subsequent electrophysiological investigation in vitro. However, the fosGFP protein revealed a similar degradation time as the endogenous c-Fos. Later development of transgenic mouse lines enabled more stable labeling of neurons expressing IEGs, allowing investigations over longer periods [8,9]. Both studies reported reactivation (IEG expression) of neurons during encoding and retrieval in many brain areas, including the hippocampus. Those early studies of IEG reactivation represented the first evidence of the existence of a memory engram.

Current Opinion in Neurobiology 2022, 75:102568

This review comes from a themed issue on **Systems Neuroscience 2022**

Edited by **Joshua Johansen** and **Laura L Colgin**

For complete overview of the section, please refer the article collection - [Systems Neuroscience 2022](#)

Available online 2 June 2022

<https://doi.org/10.1016/j.conb.2022.102568>

0959-4388/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Abbreviations

BLA, Basolateral Amygdala; CPFE, Context Preexposure Facilitation Effect; CA1, Cornus Ammonius 1; CA3, Cornus Ammonius 3; DG, Dentate Gyrus; IEGs, Immediate Early Genes; LTP, Long-Term Potentiation; LTD, Long-Term Depression.

Introduction

The question of where and how memories are encoded and retrieved has been challenging scientists and

Neves et al. (2008) conceptualized the importance of manipulating the physiology of neuronal networks active during encoding [10]. Rizzi et al. (2009) first reported their successful memory recall by optogenetically reactivating c-Fos tagged neurons. Liu et al. (2012) and Garner et al. (2012) were the first to publish similar findings [11–14]. They showed that this manipulation leads to memory retrieval. One critical factor in some of the above studies is that their optogenetic activation cannot imitate the natural sequential activation of engram cells. As mimicking the natural firing pattern of cells is challenging, another approach was taken by inhibiting neurons in the hippocampus that expressed IEGs (c-Fos or Arc) during fear conditioning and demonstrated a decrease in context-specific freezing behavior during inactivation [15,16]. These manipulation studies suggest that the neuronal activity of artificially activated IEG tagged neurons in the hippocampus plays an essential role in memory retrieval.

The third engram evidence strategy was first followed by Steinmetz et al. (1989) by performing classical conditioning of muscles reflexes [17]. The entire learning process took place intracranially by pairing the stimulation of two cerebellar regions. The restriction to intracranial stimulation only represents one out of two criteria proposed by Martin and Morris for implanting artificial memories [18]. Their second criterion expects the retrieval of the artificial implanted memory by an external event, which was accomplished by the study of Vetere et al. (2019). They took advantage of the well-known olfactory system and implanted a memory artificially via optogenetic stimulation of particular olfactory glomeruli and the aversion and reward mediating ventral tegmental area. This allowed the retrieval of this implanted memory via exposure to respective external odor stimuli [19]. On a similar note, in a study by Ramirez et al. (2013), channelrhodopsin-2 was introduced in c-Fos expressing cells during the exploration of a neutral environment [20]. Respective cells were later optogenetically activated during fear conditioning in a different environment. Yet, mice revealed subsequently increased freezing behavior in the previous neutral environment. The successful artificial memory implantation displays another support for the existence of memory engram.

While we find strong support for the existence of engrams in these studies, diverse and complex natures of memory engram cells and the content/aspect these cells are encoding remain to be elucidated. More specifically, IEG expression is mostly used as an activity marker, and respective cells are equated as engram cells. However, the functional heterogeneity of different plastic changes will remain essential to consider and might reveal a more complex nature of our memory system. Encoding any experience can lead to the storage of a broad range of different types of information such as different sensory

modalities, internally generated signals, or higher-order information integrating them. Moreover, different types of computation support those diverse inputs. Nevertheless, many engram studies testing the causal relationship using neuronal manipulation did not have a strong focus on these aspects of memory. In this review, we highlight recent studies revealing the heterogeneous nature of memory engram cells. By introducing hippocampus-dependent memory as an example, we discuss how these different memory traces might comprehensively support our memories of episodic experiences.

The complex induction and functionality of IEGs

IEGs have been used as neuronal activity markers in neuroscience fields. IEGs expression is generally low in quiescent cells but is transiently induced at the transcriptional level by extracellular stimulation [21]. As early as the 1980s, researchers have found that c-Fos is rapidly induced after electrical stimulation or administration of growth factors [22–26]. Significantly, high-frequency synaptic stimulation, which resembles the stimulation required to induce long-term potentiation (LTP), increased IEGs expression [27]. In line with previous results, Jiang et al. (2021) recently discovered that Arc-positive neurons showed increased correlated activity in hippocampal cell culture [28]. Besides directly manipulating cellular activity, IEG expression can also be induced by various extracellular signals, including growth factors, immunological and neurological signals (e.g., BDNF, IL-6), and sensory and behavioral stimulations (e.g. the studies by Gallo et al., Pfaus et al., Lanahan et al., Morgan et al., O'Donnell et al., Wheeler et al.. [29–33]). Because of these features, IEGs have provided a cellular method to label and examine the functionality of activated neurons. One of the biggest strengths of using IEGs to examine activity, compared to electrophysiology methods, is that IEG studies can provide large-scale activity analysis of multiple neurons and even multiple brain regions with simple immunohistochemical methods. For example, Wheeler et al. (2013) analyzed the c-Fos expressions of 84 brain regions during fear recall, revealing a critical thalamic-hippocampal-cortical network involved in long-term fear memory [34]. Other examples are provided by Kubik et al. (2007), who reviewed IEG evidence of hippocampal function and the subregional-specific contribution to spatial and contextual memory [35].

Alterations of gene expression underlie plastic changes in the networks in the brain and hence support memory [36]. As the first genes to undergo regulation of expression following cellular stimulation, IEGs have been proposed to function as “plasticity” markers. For example, *Arc* has been repeatedly shown to participate in molecular mechanisms of synaptic plasticity [37–40]. Consistent with this idea, *Arc* knock-out mice do not

express lasting LTP or long-term depression (LTD) in the hippocampus and are impaired for long-term memory in behavioral tasks [41] (but also see the study by Kyrke-Smith et al. [42]). Besides *Arc*, other IEGs, including *Zif-268* and *c-Fos*, have also been suggested to play a role in synaptic plasticity [29]. Although more commonly used as an activity marker rather than a plasticity marker, IEG expression is not always linked to increased cellular activities. For example, a seizure study demonstrated that high-frequency burst firings do not result in *c-Fos* expression in multiple brain areas [43]. By inhibiting medial septum input to the hippocampus, Miyashita et al. (2009) found that the behavioral induction of *Arc* transcription in CA1 and CA3 regions was abolished [44]. Because medial septal inactivation does not eliminate location-specific firing in these regions [45], their results suggested that increased cell firing is insufficient to induce *Arc* transcription. Given that increased spike rate does not always lead to IEG induction and that IEG-tagged neurons are causally linked to memory, it would be a more promising approach to examine physiological activities of the engram cells in order to determine what is encoded as memory [46]. These studies indicate that IEGs do not simply represent more or less activity of neurons.

Different IEGs should not be treated as unitary entities. One supporting evidence is that the signaling cascades leading to transcription of different IEGs differ [47]. The induction cascade of IEGs typically includes the following steps: (1) extracellular stimuli activate membrane receptors and in turn initiate a series of intracellular pathways, (2) kinases mediate the activation of transcription factors that initiate the expression of IEGs, (3) protein products of IEGs mediate the expression of downstream genes or participate in other forms of signaling [48,49]. Interestingly, different IEGs have their unique characteristics. For example, unlike *c-Fos*, *Npas4* expression was selectively induced by only membrane depolarization, while *c-Fos* could also be induced by growth factors or neurotrophins, suggesting the constitutional difference in their induction pathways [50]. Additionally, the induction kinetics of IEGs varies. Experiments using artificial synaptic stimulation demonstrate that different IEGs have different stimulus thresholds for transcriptional induction [51]. For example, the *c-Fos* induction threshold is relatively high compared to others, which provides a good signal-to-noise ratio and allows anatomical mapping of *c-Fos* positive neurons during a complex behavioral paradigm [52]. In contrast, the expression of *Zif-268* is more responsive to synaptic activities at physiological levels [53]. To further illustrate the point that IEGs should be viewed as different entities, here we compare *Arc* and *Homer 1a*. Although they share similar mechanisms for transcriptional activation (MAPK/ERK cascade) and can be expressed in the same neuronal population in the hippocampus, *Homer 1a* is slower in mRNA transcription

and is subject to additional activity-dependent regulation [54]. Even a more striking difference is their functional outcome. They play a common role in molecular trafficking but interestingly produce the opposite outcomes in synaptic plasticity. Both *Arc* and *Homer 1a* mediate endocytosis and internalize AMPA receptors to reduce their surface expression [41,55]. However, while *Homer 1a* involves homeostatic scaling of recently potentiated spines [56], *Arc* preferentially targets inactive spines to increase contrasts of synaptic weights [40]. In summary, it is oversimplified to view IEGs solely as an all-or-none biomarker due to all the differences (in induction, kinetics, and functionality) leading to the final expression, and thus, the distinct roles of IEGs in memory should also be examined carefully.

Memory engram cells are heterogeneous

To better understand the complexity in IEGs, it is important to determine a specific role of neuronal ensembles expressing each IEG. Past studies parsed out a particular IEG responding to a specific aspect of memory. The hippocampal IEGs would be a good example showing such unique contributions. For example, Jenkins et al. (2004) revealed *c-Fos* expression in the hippocampus increases when animals find a novelty in the spatial arrangement of familiar cues [48]. Rats experienced either a maze where the familiar visual cues were repositioned or a maze with familiar cues located at the same position as habituation sessions. Rats that experienced the relocated cues significantly increased *c-Fos* expression in the hippocampus and its related areas. Importantly, the hippocampal *c-Fos* did not respond to familiar cues themselves, suggesting that *c-Fos* expression most prominently responds to the novelty in the arrangement but not the novelty of elements. The *c-Fos* response in the hippocampus agrees with behavioral studies showing that hippocampal lesion leads to impairment in memory of object–location association but not of objects themselves [49,50]. Together, these findings indicate that the hippocampal *c-Fos* represents “relational” or “structural” features of the elements of experience.

In line with the notion, a series of studies using the contextual fear conditioning paradigm, including context pre-exposure facilitation effect (CPFE), revealed that the hippocampus contains conjunctive representations. Conjunctive representation refers to multiple stimulus elements combined into a single entity. Immediate shock experiments following pre-exposure to stimuli showed the hippocampus encodes co-occurrence of separated features making up the context. Importantly, a variant of the CPFE paradigm, the so-called “bucket experiment”, showed that animals associated the shock with the hippocampal contextual representation acquired during the pre-exposure session rather than with the context experienced during shock presentation [51]. Importantly, under this behavioral

paradigm, the context pre-exposure increases c-Fos expression in the dorsal hippocampus [57]. The increased c-Fos positives are shown to be memory engram cells encoding the contextual information, which can be later associated with a shock representation stored in the basolateral amygdala (BLA) [58]. In their experiment, c-Fos positives in CA1 and BLA are labeled during pre-exposure and immediate shock, respectively. Simultaneous optogenetic stimulation of these two cell populations in their home cage results in freezing behavior during the later testing session in the preexposed context. These experiments suggest that c-Fos positive cells during context pre-exposure are the conjunctive representation and satisfy a requirement as memory engram cells.

While c-Fos positive cells in the hippocampus encode contextual information as a conjunctive representation, it is not necessarily the case that other IEGs play identical roles. Experiments by Barbosa et al. (2013) showed that responses to novel relational structures differed between IEGs [59]. In the hippocampus, c-Fos expression responds to a novel location of the object, while *Zif-268* did not show such specific responses to the contextual feature. Further, it has also been reported that there is no overlap between *Homer 1a* positive cells formed during pre-exposure and *Arc* positive cells during immediate shock. This observation indicates that these two IEGs make inconsistent contributions to the formation and expression of contextual memory [60]. Another recent example showed that different IEG-expressing engram cells mediate different computations. Sun et al. (2020) demonstrated a functional heterogeneity between c-Fos-expressing and *Npas4*-expressing engram cells in the dentate gyrus (DG) during contextual fear conditioning [61]. They found that c-Fos-expressing engram cells promote memory generalization, while *Npas4*-expressing engram cells promote memory discrimination. Moreover, the two classes of engram cells receive afferent projections that were differently modified by contextual fear conditioning, suggesting the engram cells engage distinct functional circuitry. Overall, these findings suggest that neuronal ensembles characterized by the expression of different IEGs do not have interchangeable roles for memory, even within the same subregion of the hippocampus.

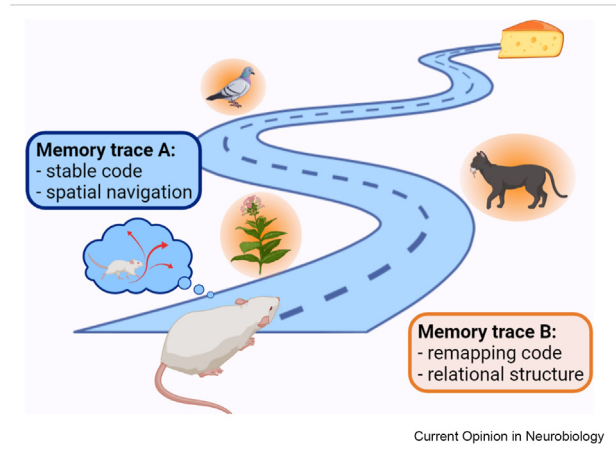
Reconciliation of hippocampal codes: A perspective from heterogeneous memory engrams

How does the heterogeneity in engram cells reconcile a coherent functioning of a specific brain structure? It is important to note that, in the hippocampus, the field has not reached a fully comprehensive view of its role in memory. Vast literature demonstrates its involvement in

spatial navigation [62–66]. This cognitive process requires the more specific computation of locale information based on sensory cues and self-movement signals to navigate the animal from the current position to another location [67–70]. Extended views posit the hippocampal computation being navigation within ‘memory space’ or ‘mental map’ that allows sequences of events to be integrated into more generalized conceptual dimensions [71,72]. In these views, a critical question is whether the hippocampus computes the navigation within the abstract space or only provides a substrate to define the relational structure of events or cues [73]. The former requires a stable map for accurate computation (but also see the study by Kinsky et al. [74]). The latter can assimilate multiple maps for the same physical space depending on the nature of the experience. Previous studies do not reject these two hypotheses. As a support for the first view, the location-specific firing of place cells becomes more stable when the animal participates in a spatial task than the spatial map of randomly foraging animals [75]. Also, in line with the navigational computation by hippocampal place cells, Ormond and O’Keefe (2021) recently reported strong modulation of place fields by vectors pointing to a goal location [76]. In contrast, various internal factors influence the hippocampal activity and produce “remapping” of place fields [77–79]. As a striking example, contextual fear conditioning caused a robust remapping of place fields even though the animal was in the same physical space [80]. These studies raise two opposing views on the hippocampal role of memory and its contribution to spatial navigation.

The apparent incompatibility of the two hypotheses can be resolved by discarding an assumption that the hippocampus has the sole role in episodic memory. Here, we propose that the hippocampus has more than two different roles for episodic memory, each supported by distinct types of memory engram cells. As opposed to the over-simplified architecture of the hippocampal trisynaptic circuit, anatomical studies elucidated more complicated and diverse connections with extra-hippocampal structures, possibly allowing flexible routing of different kinds of information (e.g. the study by Goode et al. [81]). Each hippocampal subfield is also more heterogeneous in multiple dimensions than previously thought, making various contributions to its physiology and function [82–87]. For example, a recent study found that DG represented sensory cues and spatial information in orthogonal populations of granule cells [88]. Importantly, non-homogeneous hippocampal wiring alone does not fully account for the heterogeneous memory engram cells. As we reviewed earlier, different IEGs have non-overlapping induction pathways and play diverse roles in plasticity. And different classes of engram cells characterized by different IEGs

Figure 1

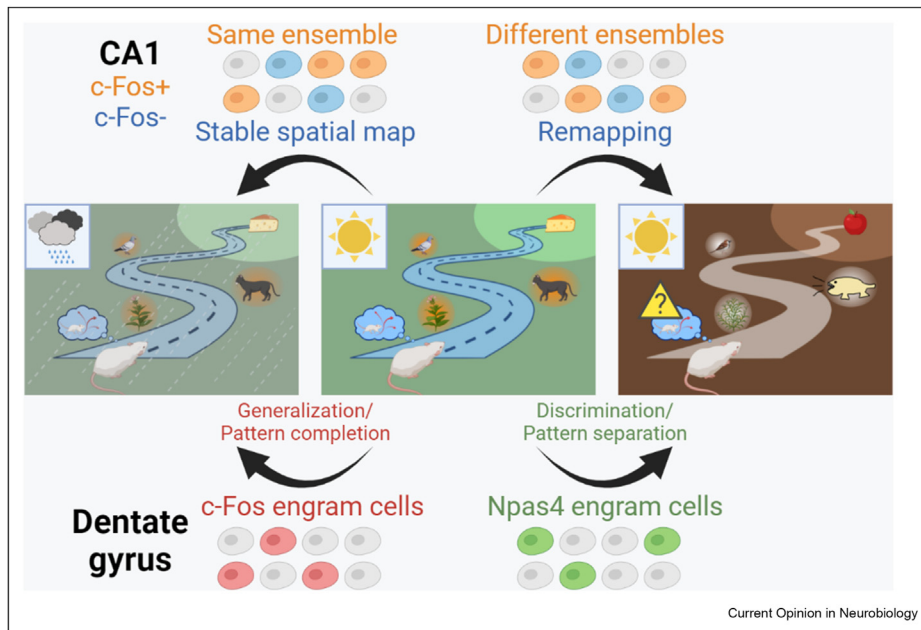


Heterogeneous memory traces in the hippocampal CA1. One type of memory trace supports spatial navigation through stable spatial maps. Another type of memory trace provides the relational structure of contextual elements in the experience.

do not always have identical roles for memory. This evidence favors a view that each memory is collectively and heterogeneously supported by different kinds of long-lasting changes [89,90].

The proposed scheme explains that spatial computation for navigation and storage of relational structure are achieved by two distinct classes of hippocampal memory engram cells. Upon exposure to a novel environment, only a fraction of place cells express c-Fos in CA1 of the hippocampus [46] (a similar observation with Arc is reported in the study by Lee *et al.* [91]). The IEG studies discussed earlier suggest these engram cells encode the relational structure of the contextual cues. Importantly, when revisiting the same environment later, c-Fos positive cells are more likely to change their firing locations, indicating their unreliability of computation for spatial navigation. On the other hand, the remaining population of place cells (c-Fos negatives) stably maintains their place fields. Notably, the formation of location-specific firing is mediated by experience-dependent hippocampal plasticity, suggesting that the c-Fos negative neurons undergo synaptic changes during the experience and thus are also a part of memory engram cells (e.g. the studies by Wilson *et al.*, Geiller *et al.* [92,93]). A recent study supports this view and found preferential thalamocortical synaptic strengthening in c-Fos negative cells in the superficial layers of the barrel cortex after the olfactory association task [94]. These results suggest that, in the hippocampus, ‘c-Fos positive engram cells’ store the relational structure of the experience, and “c-Fos negative engram cells” achieve computation of

Figure 2



Different responses and roles of distinct classes of memory engrams. In the CA1, c-Fos positive and negative pyramidal cells show different responses in their firing rates and place fields. In DG, c-Fos positive engram cells contribute to pattern completion and support context generalization, while distinct Npas4 positive engram cells play a role in pattern separation and context discrimination.

locale information for spatial navigation (Figure 1), again indicating heterogeneous neuronal ensembles to support hippocampal contribution to memory.

Conclusion

Memory engram studies, combining IEG tagging and manipulation of neuronal activity, demonstrated a causal link between the activity in a subset of neurons and a specific piece of memory. However, further investigations of IEGs and their complexity are required to unveil the mechanisms underlying memory representation, encoding, and retrieval. Different factors activate different IEGs, producing various plastic changes in the network and making distinct contributions to the mnemonic processes. In that sense, memory engram is not a unitary entity.

The use of IEGs is still a powerful approach to disentangle their specific roles for memory. For example, c-Fos expression is significantly elevated in the hippocampus when the animal found a novel arrangement in the familiar cues, supporting the role of the c-Fos ensemble for encoding relational structure in the experience [95]. Importantly, the c-Fos positive cells are a neuronal subpopulation distinct from stable place cells in the CA1 and Npas4 positive granule cells in DG, suggesting each memory engram supports different aspects of the episodic experience for later retrieval (Figure 2) [46,61].

Cooperativity across heterogeneous memory traces might explain how representational drift in the hippocampal spatial maps achieves congruency of memory over time. Contrary to the expectation that representations of memory need to be stable, long-term imaging of Ca²⁺ activity in the hippocampal neurons revealed continuous drifting in their representation of space [96–99]. Although firing locations of place cells are not stable, recent studies imply hippocampal neural codes independent of spike locations. Examples of these codes include context-specific firing rates of IEG positive cells [46,91] and synchronous activity that cannot be explained solely by overlaps of place fields [100]. If one of the representations is as stable as memory itself, functional coupling of these different representations provides reliable computation for spatial navigation while robustly retaining the hippocampal memory [101]. Further studies are required to test this prediction and understand how our brain stores memory under continuous changes of the network.

Conflict of interest statement

Nothing declared.

Acknowledgements

We appreciate Drs. Laura Colgin and Joshua Johansen for providing us with the opportunity to write this review article. We also would like to thank OIST colleagues and, in particular, our lab members for their daily support of our work. All figures are created with BioRender.com. This work was

supported by Ministry of Education, Culture, Sports, Science and Technology (MEXT) Grant-in-Aid for Scientific Research (21H02585) and institutional funding.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Semon WR: *The mneme*. Allen & Unwin; 1921.
2. Lashley KS: **Mass action in cerebral function**. *Science* 1931, **73**:245–254.
3. Lashley KS: *Brain mechanisms and intelligence: a quantitative study of injuries to the brain*. University of Chicago Press; 1929.
4. Tonegawa S, Liu X, Ramirez S, Redondo R: **Memory engram cells have come of age**. *Neuron* 2015, **87**:918–931.
5. Josselyn SA, Tonegawa S: **Memory engrams: recalling the past and imagining the future**. *Science* 2020, **367**: eaaw4325.
6. Guzowski JF, McNaughton BL, Barnes CA, Worley PF: **Environment-specific expression of the immediate-early gene Arc in hippocampal neuronal ensembles**. *Nat Neurosci* 1999, **2**:1120–1124.
7. Barth AL, Gerkin RC, Dean KL: **Alteration of neuronal firing properties after in vivo experience in a FosGFP transgenic mouse**. *J Neurosci* 2004, **24**:6466–6475.
8. Reijmers LG, Perkins BL, Matsuo N, Mayford M: **Localization of a stable neural correlate of associative memory**. *Science* 2007, **317**:1230–1233.
9. Tayler KK, Tanaka KZ, Reijmers LG, Wiltgen BJ: **Reactivation of neural ensembles during the retrieval of recent and remote memory**. *Curr Biol* 2013, **23**.
10. Neves G, Cooke SF, Bliss TVP: **Synaptic plasticity, memory and the hippocampus: a neural network approach to causality**. *Neuroscience* 2008, **16**:1536–1551.
11. Rizzi M, Powell K, Hefendehl J, Fernandes A, Häusser M: **Memory recall driven by optical stimulation of functionally identified sub-populations of neurons**. 2009. October 17–21.
12. Buchen L: **Total recall achieved**. *Nature* 2009, <https://doi.org/10.1038/news.2009.1016>.
13. Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Deisseroth K, Tonegawa S: **Optogenetic stimulation of a hippocampal engram activates fear memory recall**. *Nature* 2012, **484**:381–385.
14. Garner AR, Rowland DC, Hwang SY, Baumgaertel K, Roth BL, Kentros C, Mayford M: **Generation of a synthetic memory trace**. *Science* 2012, **335**:1513–1516.
15. Denny CA, Kheirbek MA, Alba EL, Tanaka KF, Brachman RA, Laughman KB, Tomm NK, Turi GF, Losonczy A, Hen R: **Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis**. *Neuron* 2014, **83**: 189–201.
16. Tanaka KZ, Pevzner A, Hamidi AB, Nakazawa Y, Graham J, Wiltgen BJ: **Cortical representations are reinstated by the Hippocampus during memory retrieval**. *Neuron* 2014, **84**: 347–354.
17. Steinmetz JE, Lavond DG, Thompson RF: **Classical conditioning in rabbits using pontine nucleus stimulation as a conditioned stimulus and inferior olive stimulation as an unconditioned stimulus**. *Synapse* 1989, **3**:225–233.
18. Martin SJ, Morris RGM: **New life in an old idea: the synaptic plasticity and memory hypothesis revisited**. *Hippocampus* 2002, **12**:609–636.
19. Vetere G, Tran LM, Moberg S, Steadman PE, Restivo L, Morrison FG, Ressler KJ, Josselyn SA, Frankland PW: **Memory**

- formation in the absence of experience. *Nat Neurosci* 2019, **22**:933–940.
20. Ramirez S, Liu X, Lin P-A, Suh J, Pignatelli M, Redondo RL, Ryan TJ, Tonegawa S: **Creating a false memory in the hippocampus.** *Science* 2013, **341**:387–391.
 21. Sheng M, Greenberg ME: **The regulation and function of c-fos and other immediate early genes in the nervous system.** *Neuron* 1990, **4**:477–485.
 22. Sagar SM, Sharp FR, Curran T: **Expression of c-fos protein in brain: metabolic mapping at the cellular level.** *Science* 1988, **240**:1328–1331.
 23. Greenberg ME, Ziff EB: **Stimulation of 3T3 cells induces transcription of the c-fos proto-oncogene.** *Nature* 1984, **311**:433–438.
 24. Müller R, Bravo R, Burckhardt J, Curran T: **Induction of c-fos gene and protein by growth factors precedes activation of c-myc.** *Nature* 1984, **312**:716–720.
 25. Kruijer W, Cooper JA, Hunter T, Verma IM: **Platelet-derived growth factor induces rapid but transient expression of the c-fos gene and protein.** *Nature* 1984, **312**:711–716.
 26. Cochran BH, Zullo J, Verma IM, Stiles CD: **Expression of the c-fos gene and of an fos-related gene is stimulated by platelet-derived growth factor.** *Science* 1984, **226**:1080–1082.
 27. Cole AJ, Saffen DW, Baraban JM, Worley PF: **Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation.** *Nature* 1989, **340**:474–476.
 28. Jiang Y, VanDongen AMJ: **Selective increase of correlated activity in Arc-positive neurons after chemically induced long-term potentiation in cultured hippocampal neurons.** *eNeuro* 2021, <https://doi.org/10.1523/ENEURO.0540-20.2021>.
 29. Gallo FT, Katche C, Morici JF, Medina JH, Weisstaub NV: **Immediate early genes, memory and psychiatric disorders: focus on c-Fos, Egr1 and Arc.** *Front Behav Neurosci* 2018, **12**:79.
 30. Pfaus JG, Heeb MM: **Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents.** *Brain Res Bull* 1997, **44**:397–407.
 31. Lanahan A, Worley P: **Immediate-early genes and synaptic function.** *Neurobiol Learn Mem* 1998, **70**:37–43.
 32. Morgan JL, Curran T: **Stimulus-transcription coupling in the nervous-system - involvement of the inducible proto-oncogenes fos and jun.** *Annu Rev Neurosci* 1991, **14**:421–451.
 33. O'Donnell A, Odrowaz Z, Sharrocks AD: **Immediate-early gene activation by the MAPK pathways: what do and don't we know?** *Biochem Soc Trans* 2012, **40**:58–66.
 34. Wheeler AL, Teixeira CM, Wang AH, Xiong X, Kovacevic N, Lerch JP, McIntosh AR, Parkinson J, Frankland PW: **Identification of a functional connectome for long-term fear memory in mice.** *PLoS Comput Biol* 2013, **9**, e1002853.
 35. Kubik S, Miyashita T, Guzowski JF: **Using immediate-early genes to map hippocampal subregional functions.** *Learn Mem* 2007, **14**:758–770.
 36. Tischmeyer W, Grimm R: **Activation of immediate early genes and memory formation.** *Cell Mol Life Sci* 1999, **55**:564–574.
 37. Chowdhury S, Shepherd JD, Okuno H, Lyford G, Petralia RS, Plath N, Kuhl D, Huganir RL, Worley PF: **Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking.** *Neuron* 2006, **52**:445–459.
 38. Shepherd JD, Rumbaugh G, Wu J, Chowdhury S, Plath N, Kuhl D, Huganir RL, Worley PF: **Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors.** *Neuron* 2006, **52**:475–484.
 39. Messaoudi E, Kanhema T, Soulé J, Tiron A, Dageyte G, da Silva B, Bramham CR: **Sustained Arc/Arg3.1 synthesis controls long-term potentiation consolidation through regulation of local actin polymerization in the dentate gyrus in vivo.** *J Neurosci* 2007, **27**:10445–10455.
 40. Okuno H, Akashi K, Ishii Y, Yagishita-Kyo N, Suzuki K, Nonaka M, Kawashima T, Fujii H, Takemoto-Kimura S, Abe M, *et al.*: **Inverse synaptic tagging of inactive synapses via dynamic interaction of Arc/Arg3.1 with CaMKII β .** *Cell* 2012, **149**:886–898.
 41. Plath N, Ohana O, Dammernann B, Errington ML, Schmitz D, Gross C, Mao X, Engelsberg A, Mahlke C, Welzl H, *et al.*: **Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories.** *Neuron* 2006, **52**:437–444.
 42. Kyrke-Smith M, Volk LJ, Cooke SF, Bear MF, Huganir RL, Shepherd JD: **The immediate early gene Arc is not required for hippocampal long-term potentiation.** *J Neurosci* 2021, **41**:4202–4211.
 43. Labiner DM, Butler LS, Cao Z, Hosford DA, Shin C, McNamara JO: **Induction of c-fos mRNA by kindled seizures: complex relationship with neuronal burst firing.** *J Neurosci* 1993, **13**:744–751.
 44. Miyashita T, Kubik S, Haghghi N, Steward O, Guzowski JF: **Rapid activation of plasticity-associated gene transcription in hippocampal neurons provides a mechanism for encoding of one-trial experience.** *J Neurosci* 2009, **29**:898–906.
 45. Mizumori SJ, McNaughton BL, Barnes CA, Fox KB: **Preserved spatial coding in hippocampal CA1 pyramidal cells during reversible suppression of CA3c output: evidence for pattern completion in hippocampus.** *J Neurosci* 1989, **9**:3915–3928.
 46. Tanaka KZ, He H, Tomar A, Niisato K, Huang AJY, McHugh TJ: **The hippocampal engram maps experience but not place.** *Science* 2018, **361**:392–397.
 47. Okuno H: **Regulation and function of immediate-early genes in the brain: beyond neuronal activity markers.** *Neurosci Res* 2011, **69**:175–186.
 48. Sommerlandt FMJ, Brockmann A, Rössler W, Spaethe J: **Immediate early genes in social insects: a tool to identify brain regions involved in complex behaviors and molecular processes underlying neuroplasticity.** *Cell Mol Life Sci* 2019, **76**:637–651.
 49. Kim S, Kim H, Um JW: **Synapse development organized by neuronal activity-regulated immediate-early genes.** *Exp Mol Med* 2018, **50**:1–7.
 50. Lin Y, Bloodgood BL, Hauser JL, Lapan AD, Koon AC, Kim T-K, Hu LS, Malik AN, Greenberg ME: **Activity-dependent regulation of inhibitory synapse development by Npas4.** *Nature* 2008, **455**:1198–1204.
 51. Abraham WC, Mason SE, Demmer J, Williams JM, Richardson CL, Tate WP, Lawlor PA, Dragunow M: **Correlations between immediate early gene induction and the persistence of long-term potentiation.** *Neuroscience* 1993, **56**:717–727.
 52. Zhu XO, Brown MW, McCabe BJ, Aggleton JP: **Effects of the novelty or familiarity of visual stimuli on the expression of the immediate early gene c-fos in rat brain.** *Neuroscience* 1995, **69**:821–829.
 53. Worley PF, Bhat RV, Baraban JM, Erickson CA, McNaughton BL, Barnes CA: **Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement.** *J Neurosci* 1993, **13**:4776–4786.
 54. Vazdarjanova A, McNaughton BL, Barnes CA, Worley PF, Guzowski JF: **Experience-dependent coincident expression of the effector immediate-early genes arc and Homer 1a in hippocampal and neocortical neuronal networks.** *J Neurosci* 2002, **22**:10067–10071.
 55. Clifton NE, Trent S, Thomas KL, Hall J: **Regulation and function of activity-dependent homer in synaptic plasticity.** *Mol Neuropsychiatry* 2019, **5**:147–161.
 56. Okada D, Ozawa F, Inokuchi K: **Input-specific spine entry of soma-derived Ves1-1S protein conforms to synaptic tagging.** *Science* 2009, **324**:904–909.
 57. Heroux NA, Horgan CJ, Pinizzotto CC, Rosen JB, Stanton ME: **Medial prefrontal and ventral hippocampal contributions to**

- incidental context learning and memory in adolescent rats.** *Neurobiol Learn Mem* 2019, **166**:107091.
58. Ohkawa N, Saitoh Y, Suzuki A, Tsujimura S, Murayama E, Kosugi S, Nishizono H, Matsuo M, Takahashi Y, Nagase M, *et al.*: **Artificial association of pre-stored information to generate a qualitatively new memory.** *Cell Rep* 2015, <https://doi.org/10.1016/j.celrep.2015.03.017>.
59. Barbosa FF, Santos JR, Meurer YSR, Macêdo PT, Ferreira LMS, Pontes IMO, Ribeiro AM, Silva RH: **Differential cortical c-fos and zif-268 expression after object and spatial memory processing in a standard or episodic-like object recognition task.** *Front Behav Neurosci* 2013, **7**:112.
60. Pevzner A, Guzowski JF: *Immediate-early gene transcriptional activation in hippocampus CA1 and CA3 does not accurately reflect rapid, pattern completion-based retrieval of context memory.* **22**; 2014:1–5.
61. Sun X, Bernstein MJ, Meng M, Rao S, Sørensen AT, Yao L, Zhang X, Anikeeva PO, Lin Y: **Functionally distinct neuronal ensembles within the memory engram.** *Cell* 2020, **181**: 410–423.e17.
- By labeling the c-Fos and Npas4 memory engrams in the Dentate gyrus, this study showed that functionally distinct neuronal ensembles promote different processes, generalization and discrimination, for contextual memory.
62. Moser EI, Kropff E, Moser M-B: **Place cells, grid cells, and the brain's spatial representation system.** *Annu Rev Neurosci* 2008, **31**:69–89.
63. Morris RG: **Distinctive computations and relevant associative processes: hippocampal role in processing, retrieval, but not storage of allocentric spatial memory.** *Hippocampus* 1991, **1**: 287–290.
64. Eichenbaum H: **The role of the hippocampus in navigation is memory.** *J Neurophysiol* 2017, **117**:1785–1796.
65. O'Keefe J, Burgess N, Donnett JG, Jeffery KJ, Maguire EA: **Place cells, navigational accuracy, and the human hippocampus.** *Philos Trans R Soc Lond B Biol Sci* 1998, **353**:1333–1340.
66. Buzsáki G, Moser EI: *Memory, navigation and theta rhythm in the hippocampal-entorhinal system.* **16**; 2013:130–138.
67. Whishaw IQ: **Place learning in hippocampal rats and the path integration hypothesis.** *Neurosci Biobehav Rev* 1998, **22**: 209–220.
68. McNaughton BL, Battaglia FP, Jensen O, Moser EI, Moser M-B: **Path integration and the neural basis of the “cognitive map.”** *Nat Rev Neurosci* 2006, **7**:663–678.
69. Jayakumar RP, Madhav MS, Savelli F, Blair HT, Cowan NJ, Knierim JJ: **Recalibration of path integration in hippocampal place cells.** *Nature* 2019, **566**:533–537.
- Using a motion-adapting augmented-reality dome, this study established an enduring conflict between self-movement and (adapting) landmarks. From the activity of place cells during the task, they found recalibration of the path-integration gain. This study beautifully elucidated the computational processes of path integration, and their result argues against the path-integration gain as a constant but rather represents high plasticity.
70. Burgess N: **Spatial memory: how egocentric and allocentric combine.** *Trends Cognit Sci* 2006, **10**:551–557.
71. Eichenbaum H: **The hippocampus and mechanisms of declarative memory.** *Behav Brain Res* 1999, **103**:123–133.
72. Miliivojevic B, Doeller CF: **Mnemonic networks in the hippocampal formation: from spatial maps to temporal and conceptual codes.** *J Exp Psychol Gen* 2013, **142**:1231–1241.
73. Eichenbaum H, Cohen NJ: **Can we reconcile the declarative memory and spatial navigation views on hippocampal function?** *Neuron* 2014, **83**:764–770.
74. Kinsky NR, Sullivan DW, Hasselmo ME, Mau W, Eichenbaum HB, Eichenbaum HB: **Hippocampal place fields maintain a coherent and flexible map across long timescales.** *Curr Biol* 2018, **28**:3578–3588.e6.
75. Kentros CG, Agnihotri NT, Streater S, Hawkins RD, Kandel ER: **Increased attention to spatial context increases both place field stability and spatial memory.** *Neuron* 2004, **42**:283–295.
76. Ormond J, O'Keefe J: **Hippocampal place cells use vector computations to navigate.** *bioRxiv* 2021, <https://doi.org/10.1101/2021.06.23.449621>.
- Using the honeycomb maze, which allows examination of place cell activity during a choice of trajectories, the authors found strong modulation of the activity pointing towards the goal location. This property of place cells enables the calculation of optimal trajectories from different locations of the animal.
77. Smith DM, Mizumori SJY: **Hippocampal place cells, context, and episodic memory.** *Hippocampus* 2006, **16**:716–729.
78. Colgin LL, Moser EI, Moser MB: **Understanding memory through hippocampal remapping.** *Trends Neurosci* 2008, **31**: 469–477.
79. Kubie JL, Levy ERJ, Fenton AA: **Is hippocampal remapping the physiological basis for context?** *Hippocampus* 2020, **30**: 851–864.
80. Moita MAP, Rosis S, Zhou Y, LeDoux JE, Blair HT: **Putting fear in its place: remapping of hippocampal place cells during fear conditioning.** *J Neurosci* 2004, **24**:7015–7023.
81. Goode TD, Tanaka KZ, Sahay A, McHugh TJ: **An integrated index: engrams, place cells, and hippocampal memory.** *Neuron* 2020, **107**:805–820.
82. Fanselow MS, Dong H-W: **Are the dorsal and ventral hippocampus functionally distinct structures?** *Neuron* 2010, **65**: 7–19.
83. Sharif F, Tayebi B, Buzsáki G, Royer S, Fernandez-Ruiz A: **Subcircuits of deep and superficial CA1 place cells support efficient spatial coding across heterogeneous environments.** *Neuron* 2021, **109**:363–376.e6.
84. Navas-Olive A, Valero M, Jurado-Parras T, de Salas-Quiroga A, Averkin RG, Gambino G, Cid E, de la Prida LM: **Multimodal determinants of phase-locked dynamics across deep-superficial hippocampal sublayers during theta oscillations.** *Nat Commun* 2020, **11**:2217.
- The study identified various factors influencing the spike timing of CA1 pyramidal cells during theta oscillation. Remarkably, these factors, revealed by both unbiased computational models and experimental data, have different impacts on deep and superficial layers of pyramidal cells, suggesting distinct roles and computations between these sublayers.
85. Soltesz I, Losonczy A: **CA1 pyramidal cell diversity enabling parallel information processing in the hippocampus.** *Nat Neurosci* 2018, **21**:484–493.
86. Nakazawa Y, Pevzner A, Tanaka KZ, Wiltgen BJ: **Memory retrieval along the proximodistal axis of CA1.** *Hippocampus* 2016, <https://doi.org/10.1002/hipo.22596>.
87. Igarashi KM, Ito HT, Moser EI, Moser M-B: **Functional diversity along the transverse axis of hippocampal area CA1.** *FEBS Lett* 2014, **588**:2470–2476.
88. Tuncdemir SN, Grosmark AD, Turi GF, Shank A, Bowler JC, Ordek G, Losonczy A, Hen R, Lacefield CO: **Parallel processing of sensory cue and spatial information in the dentate gyrus.** *Cell Rep* 2022, **38**:110257.
89. Tanaka KZ: **Heterogeneous representations in the hippocampus.** *Neurosci Res* 2021, **165**:1–5.
90. Tanaka KZ, McHugh TJ: **The hippocampal engram as a memory index.** *J Exp Neurosci* 2018, **12**. 1179069518815942.
91. Lee BH, Shim JY, Moon HC, Kim DW, Kim J, Yook JS, Kim J, Park HY: **Real-time visualization of mRNA synthesis during memory formation in live animals.** *bioRxiv* 2021, <https://doi.org/10.1101/2021.09.01.458476>.
- This paper introduces for the first time a genetically-encoded RNA indicator (GERI) that allows live in vivo imaging of the IEG Arc expression in mice brains. Combined with calcium imaging, they showed that only a subset of CA1 neurons expressed Arc during encoding and revealed their context-specific calcium activity.
92. Wilson MA, McNaughton BL: **Dynamics of the hippocampal ensemble code for space.** *Science* 1993, **261**:1055–1058.

93. Geiller T, Sadeh S, Rolotti SV, Blockus H, Vancura B, Negrean A, Murray AJ, Rózsa B, Polleux F, Clopath C, *et al.*: **Local circuit amplification of spatial selectivity in the hippocampus.** *Nature* 2021, <https://doi.org/10.1038/s41586-021-04169-9>.

This study combined monosynaptic retrograde tracing and single-cell optogenetic manipulation to investigate local CA1 circuits during navigation. Single place cells amplify spatial selectivity by recruiting functionally related subnetworks, indicating plastic changes are involved in forming place fields.

94. Lee J, Urban-Ciecko J, Park E, Zhu M, Myal SE, Margolis DJ, Barth AL: **FosGFP expression does not capture a sensory learning-related engram in superficial layers of mouse barrel cortex.** *Proc Natl Acad Sci U S A* 2021, **118**.
95. Jenkins TA, Amin E, Pearce JM, Brown MW, Aggleton JP: **Novel spatial arrangements of familiar visual stimuli promote activity in the rat hippocampal formation but not the parahippocampal cortices: a c-fos expression study.** *Neuroscience* 2004, **124**:43–52.
96. Ziv Y, Burns LD, Cocker ED, Hamel EO, Ghosh KK, Kitch LJ, El Gamal A, Schnitzer MJ: **Long-term dynamics of CA1 hippocampal place codes.** *Nat Neurosci* 2013, **16**:264–266.
97. Rubin A, Geva N, Sheintuch L, Ziv Y: **Hippocampal ensemble dynamics timestamp events in long-term memory.** *Elife* 2015, **4**.
98. Sheintuch L, Geva N, Baumer H, Rechavi Y, Rubin A, Ziv Y: **Multiple maps of the same spatial context can stably coexist in the mouse Hippocampus.** *Curr Biol* 2020, **30**:1467–1476.e6.
- Long-term calcium imaging of CA1 neurons during exposure to the same environment revealed multiple maps for a particular environment without discernible external changes.
99. Attardo A, Lu J, Kawashima T, Okuno H, Fitzgerald JE, Bito H, Schnitzer MJ: **Long-term consolidation of ensemble neural plasticity patterns in hippocampal area CA1.** *Cell Rep* 2018, **25**:640–650.e2.
100. Levy ERJ, Park EH, Redman WT, Fenton AA: **A neuronal code for space in hippocampal coactivity dynamics independent of place fields.** *bioRxiv* 2021, <https://doi.org/10.1101/2021.07.26.453856>.
- This study identified a coactivity coding scheme in the CA1 of the hippocampus. Importantly, their coactivity is independent of the location-specific firing of the place cells. Nevertheless, the coactivity was as reliable as the place fields when discriminating environments.
101. Trouche S, Perestenko PV, Ven GM van de, Bratley CT, McNamara CG, Campo-Urriza N, Black SL, Reijmers LG, Dupret D: **Recoding a cocaine-place memory engram to a neutral engram in the hippocampus.** *Nat Neurosci* 2016, **19**:564–567.